Measurement of Spatiotemporal Intracellular Deformation of Cells Adhered to Collagen Matrix During Freezing of Biomaterials

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Preservation of structural integrity inside cells and at cell-extracellular matrix (ECM) interfaces is a key challenge during freezing of biomaterials. Since the post-thaw functionality of cells depends on the extent of change in the cytoskeletal structure caused by complex cell-ECM adhesion, spatiotemporal deformation inside the cell was measured using a newly developed microbead-mediated particle tracking deformetry (PTD) technique using fibroblast-seeded dermal equivalents as a model tissue. Fibronectin-coated 500 nm diameter microbeads were internalized in cells, and the microbead-labeled cells were used to prepare engineered tissue with type I collagen matrices. After a 24 h incubation the engineered tissues were directionally frozen, and the cells were imaged during the process. The microbeads were tracked, and spatiotemporal deformation inside the cells was computed from the tracking data using the PTD method. Effects of particle size on the deformation measurement method were tested, and it was found that microbeads represent cell deformation to acceptable accuracy. The results showed complex spatiotemporal deformation patterns in the cells. Large deformation in the cells and detachments of cells from the ECM were observed. At the cellular scale, variable directionality of the deformation was found in contrast to the one-dimensional deformation pattern observed at the tissue scale, as found from earlier studies. In summary, this method can quantify the spatiotemporal deformation in cells and can be correlated to the freezing-induced change in the structure of cytoplasm and of the cell-ECM interface. As a broader application, this method may be used to compute deformation of cells in the ECM environment for physiological processes, namely cell migration, stem cell differentiation, vasculogenesis, and cancer metastasis, which have relevance to quantify mechanotransduction. [DOI: 10.1115/1.4026180]

1 Introduction

Freezing of biomaterials is adopted in a wide variety of biomedical applications including cryopreservation of native and engineered tissues [1], preservation of biospecimens [2], decellularization of native tissues for scaffold engineering [3,4], and cryosurgery of diseased tissues [5]. Although the objectives of these cryomedicine applications are diverse, it is commonly critical to understand the effects and consequences of freeze/thaw (F/T) on the functionality of tissues and biomaterials. However, the mechanism of the freezing-induced biophysical interactions within tissues and biomaterials is not fully understood yet, and many of the freezing protocols were developed empirically and tissue-type specifically. Quantitative knowledge of freezing-induced biophysical phenomena will be useful to design successful cryomedicine applications. Although post-thaw cell viability [6] has been the primary target of these applications, it has recently been recognized that for tissues, where cells are embedded in a complex three-dimensional extracellular matrix (ECM), other features beyond viability are also important to the functionality of biomaterials. This includes the microstructure of the ECM, state of the cell-matrix adhesion, and the cytoskeletal structure and organization [7–10].

Freeze/thaw of biomaterials has been reported to induce microstructural changes in the extracellular matrix due to interstitial ice

formation [8,11,12]. This tissue-level microstructural damage has been investigated using multiphoton-induced autofluorescence and second harmonic generation microscopy [13], magnetic resonance imaging [14], and histological analysis [10,15]. Though successful preservation of microstructures was reported for aortic and pulmonary valves [8] and articular cartilages [16], significant change in tissue functionality, as well as in structural and mechanical properties was also observed in other types of tissues [17–19]. To explain these microstructural changes, it was proposed that they result from complex cell-fluid-matrix interactions during freezing [7,20,21]. These studies were based on a hypothesis that biological tissues can be approximated as a poroelastic material that consists of fibrous collagen ECM saturated with interstitial fluid. These studies suggest that the volumetric expansion associated with the freezing of the interstitial fluid induces complex interactions among the ECM, fluid and cells, and these interactions result in the enlarged pore structure of the ECM and extrusion of the interstitial fluid post-thaw [7,21–23]. A measurement technique was developed [23] to quantify spatiotemporal deformation inside the ET, and these studies found that ECM deformation during freezing can be correlated to the change in ECM microstructure post-thaw [7,20].

Recently, it has been proposed that the cells play an important role in these interactions by providing structural support to the ECM and mitigating the post-thaw structural changes of the ECM. This protective role of the cells is thought to be associated with increased structural strength by cell-matrix adhesion and the strength of the cytoskeleton [24]. This finding is potentially very useful to design cryomedicine applications by (i) providing a new

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way to preserve functional tissues with a reduced amount of toxic cryoprotective agents and (ii) controlling and modulating the hierarchical porous structures (i.e., ECM and cytoskeleton) mechanistically during freezing, which may enable decellularization with minimal structural change of the ECM during freezing. In order to demonstrate this cell-mediated structural support, it is crucial to quantify the spatiotemporal deformation of the cells attached to the ECM during the freezing-induced complex cell-fluid-matrix interactions. However, it is very difficult to measure these cellular and sub-cellular deformations (i.e., small length scales) within very short time intervals (i.e., small time scales). Currently available deformation measurement techniques have been developed to quantify tissue and ECM-level deformation [23] or to assess traction force distribution on the cell periphery during slow processes, such as matrix remodeling and migration [25,26]. Thus, a new measurement method is necessary that is capable of quantifying the rapid cellular/subcellular deformation.

The objective of the present study is to develop a technique to measure the intracellular deformation of cells adhered to a collagen matrix. The technique is then applied to quantify freezing-induced spatiotemporal mechanical loading on the cells adhered to the collagen matrix. As a model tissue, human dermal fibroblasts labeled with fluorescent nanoparticles were seeded on type I collagen matrix and exposed to freezing. During freezing, the movement of intracellular fluorescent nanoparticles was imaged and further analyzed to determine spatiotemporal deformation of the cytoplasm. The results are discussed to establish a mechanistic understanding of freezing-induced biophysical processes around and inside cells embedded within the ECM.

2 Theoretical Background

In order to characterize the spatiotemporal intracellular deformation, the first and second invariants of the deformation tensors are determined as described elsewhere [27]. The underlying rationale is that invariants, which are independent of the direction, are more relevant to quantify the magnitude of the intracellular deformation of the attached cells than the directional strains [28] since the intracellular deformation results from both affine and nonaffine deformation with respect to the ECM deformation [29,30].

Fig. 1 (a) Triangular element deforms from an initial ($t = 0$) to a later configuration (at time t) with accompanied translation and rotation. The initial and final configurations are used to compute the deformation gradient tensor. (b) Regional deformation analysis scheme to delineate deformation from translation and rotation. Deformation gradient tensor $\mathbf{F}$, left Cauchy-Green tensor $\mathbf{B}$ and finally the stretch ratios λ_1 and λ_2 are computed sequentially during the method. The stretch ratios are used to compute the first invariant I_1 and second invariant I_2 .

The invariants were computed by analyzing the deformation of discretized triangular regions of the intracellular space as shown in Fig. 1(a). The vertices of this triangular region are initially located at $[X_i(0), Y_i(0)] (i = 1, 2, 3)$ at time $t = 0$. This region deforms so that its vertices move to $[X_i(t), Y_i(t)]$ at time t . The element is assumed to be planar and homogeneous while it deforms. The deformation gradient tensor ($\mathbf{F}$) can be computed from the initial and final locations of the vertices by a regional deformation analysis described elsewhere [31]. Then, the Left Cauchy-Green tensor ($\mathbf{B}$) can be subsequently determined from the deformation gradient tensor ($\mathbf{F}$) to obtain the principal stretch ratios λ_1 and λ_2 , which are the measure of deformation along the principal axes of the triangular region. These stretch ratios are used to further calculate the first invariant $I_1 = \lambda_1^2 + \lambda_2^2$ and the second invariant $I_2 = \lambda_1^2 \lambda_2^2$. The eigenvectors of $\mathbf{B}$ represent the directions of the principal stretch ratios. The procedure is summarized in Fig. 1(b).

3 Materials and Methods

3.1 Cell Culture and Reagents. Human dermal fibroblasts were cultured in Dulbecco's modified Eagle medium (DMEM/F12, Invitrogen, Grand Island, NY) supplemented with 10% fetal bovine serum, 2 mM L-glutamine, and 100 $\mu\text{g/ml}$ penicillin/streptomycin. The fibroblasts were maintained in 75 cm^2 T-flasks at 37 $^\circ\text{C}$ and 5% CO_2 . By using 0.05% trypsin with 0.53 mM EDTA, the cells were consistently harvested at 80%–90% confluence between the sixth and fifteenth passages.

3.2 Tissue Equivalents With Fluorescent Particle-Labeled Cells. Cells were labeled by internalizing fluorescent microbeads (Thermoscientific, CA, USA) according to the protocol developed earlier [32]. Briefly, the required number of cells was cultured in a petri dish for a minimum of 1 h to attach the cells. 4 ml bare DMEM was mixed with 30 μg human plasma protein fibronectin (Invitrogen) and 40 μl of 500 nm diameter fluorescent polystyrene microbead stock solution containing 1.5×10^{11} particles/ml (Thermoscientific). The mixture was kept at 37 $^\circ\text{C}$ for 10 min. This aliquot was applied on the attached cells and incubated for 3 h at 37 $^\circ\text{C}$. The microbeads were internalized by the protocol developed previously [32–35]. In these studies, it was confirmed that the beads up to 1 μm in diameter could be internalized and were not bound to the cell membrane. After incubation, the cells were rinsed with fresh medium. The cells were then trypsinized and used to prepare the engineered tissues. The cells were labeled with another smaller size particle, 20 nm diameter quantum dots (Qtracker 655, Invitrogen, Carlsbad, CA), according to the protocol described earlier [23].

Engineered tissues (ETs) were prepared after modification of the method described previously [7]. The ETs were made by polymerizing approximately 150 μl of collagen solution formed according to the method described elsewhere [23] in custom-made circular containers so that the final ET became 9 mm in diameter and approximately 1 mm in thickness. Microbead-labeled fibroblasts were seeded on the polymerized collagen gel. After the cells were attached, the ETs were cultured in supplemented medium for 24 h before the freezing procedure. The cell viability of microbead-labeled cells 24 h after the cells were seeded in the ET was $91.8 \pm 3.8\%$, as found from a membrane integrity assay.

To study the effect of particle size on the intracellular deformation measurement, cells labeled with microbeads and quantum dots (QDs) were separately used to prepare engineered tissue (cell seeding density = 2×10^5 cells/ml and collagen concentration = 3 mg/ml) as described earlier [23]. After 24 h the cells were well attached to the collagen gel.

3.3 Imaging and Analysis for Intracellular Particle Motion. Cells labeled with microbeads and QDs were imaged at 30 s intervals for 2 h using an Olympus IX71 microscope (Olympus, PA) and a Retiga Q-Imaging camera (Retiga, Surrey,

Fig. 2 Imposed temperature history characterization at location of interest. Temperature history for the present study was matched ($n=3$) with the average temperature history at $x=2$ mm, performed with a traditional directional freezing stage [23].

Canada) at $40\times$ magnification inside an incubation stage. The movement of microbeads or QDs inside the cells was tracked using image-processing software (Metamorph, CA).

From the spatiotemporal tracking data, the inter-particle distances for both types of particles were computed for several pairs of particles. This inter-particle distance $\vec{r}_i(t) - \vec{r}_j(t)$ was normalized by an initial inter-particle distance $\vec{r}_i(0) - \vec{r}_j(0)$, where i and j are indices for a pair of particles. Also the angle $\phi_{ij}(t)$ made by the line joining the two particles with a fixed reference line was computed and normalized by the initial angle $\phi_{ij}(0)$ to quantify normalized inter-particle angular orientation.

3.4 Directional Freezing of Engineered Tissue. To study the freezing-induced intracellular deformation in cells, ETs were directionally frozen on a cryostage (Linkam MDS 600, UK). The gels, prepared in custom-made containers, were carefully transferred into a quartz crucible. For the directional freezing, the quartz crucible was placed 2 mm offset to the silver base of a cryostage. The cryostage was cooled in a way to match the temperature history of ETs at the location of interest with the temperature history at $x=2$ mm obtained by a traditional directional freezing stage as shown in Fig. 2 [23].

3.5 Intracellular Particle Tracking Deformetry. ETs were imaged during freezing using a fluorescence microscope (Olympus BX51, Melville, NY) with a high-speed CMOS camera (Hispec I, Fastec Imaging, Indianapolis, IN) at $40\times$ magnification and at 10 ms intervals. The freezing and imaging procedure was controlled through a LabVIEW VI programmed to correlate the time-lapse imaging with measured temperature at the cryostage. The captured images from high-speed imaging were post-processed using ImageJ, and the locations of microbeads (i.e., $X(t)$ and $Y(t)$ positions for each microbead) were tracked by image-processing software (Metamorph, CA). These location data were smoothed by rejecting any outliers which are associated with artifacts during particle tracking. Subsequently, using the 2D tracking data, the cell (a representative cell) shown in Fig. 3(a) was discretized into triangular elements using the Delaunay algorithm, shown in Fig. 3(b). Triangles that were not part of the cell, or which were very thin characterized by high aspect ratio, were rejected. The aspect ratio of the accepted triangles was less than 2 ($n=24$ for the representative cell). The spatiotemporal deformation of each triangular element was quantified by computing the invariants using the method described in the Theoretical Background. Through this analysis the substrate rotation and translation were separated, and only the local intracellular deformation was delineated.

Fig. 3 (a) Fluorescent microbead-labeled cell attached on polymerized collagen gel. The dotted line shows the location of the freeze front at a given time as it propagates gradually from left towards right. (b) Triangular meshes have been generated using the Delaunay algorithm using the tracks of the microbeads. Some triangles are rejected based on the criteria stated in the main text. The microbeads are tracked during postprocessing after the cell deformation experiment.

4 Results

Figure 4 shows the time-lapse images of cells labeled with microbeads 4(a) and QDs 4(b). During the imaging, the microbeads remain relatively stationary in the intracellular space. On the contrary, QDs move through the intracellular space without external deformation of the cell. Representative tracks of both types of particles over 2 h are presented in Figs. 4(c) and 4(d). Although translational motion is noted, probably due to overall movement of cell and microscopic stage, no significant changes in inter-particle distance and inter-particle angular orientation are observed for the microbeads. But for the QDs the pathlines are more random and follow a zig-zag pattern with rapid erratic movements at small scale. Normalized inter-particle distance and inter-particle orientation for these pairs of particles are shown in Figs. 4(e) and 4(f), respectively. For microbeads, the normalized inter-particle distance remains closed to 1, confirming that the particles maintain approximately the same position with respect to each other. For QDs, the normalized inter-particle distance is substantially variable confirming that the QDs come close to each other and then move back to farther distances. Similar results are observed for inter-particle angular orientation. These results suggest that the microbeads are big enough not to move through the cytoskeletal structure. Therefore, movement of the microbeads closely follows the mechanical deformation of the cytoplasm.

In Fig. 5, intracellular deformation for two representative cells is presented in (a) and (b), respectively. The cells deform as the freeze front propagates (arrow in (i)), and the deformation can be visualized from fluorescence images (i), (ii), and (iii). The spatial distribution of I_1 at an intermediate time is presented in (iv). High deformation in the cell during the process is characterized by a higher value of I_1 . Spatial distribution of I_2 is presented in (v). The distribution of the two invariants is different in the cell. The directional variability of cell deformation is presented in (vi). The

Fig. 4 Movement of nanoparticles inside the cell. For microbeads the relative position of the particles remains relatively constant (two representative particles are marked by arrows) over a 2 h time interval as shown in (a). For QDs (two representative particles are marked by arrows) the relative positions between particles change over time as shown in (b). Loci of the two representative particles over 2 h are presented in (c) for microbeads and (d) for QDs. The initial positions (at $t = 0$) of the nanoparticles are marked by the arrows. For microbeads, the loci are pathlines of two particles although they look like points implying very small movement of the particles. For the same representative pair of nanoparticles, the normalized inter-particle distance $r(t)/r(t=0)$ with time is presented in (e). The value remains close to 1 for microbeads but varies substantially for QDs. The same result is observed for inter-particle angular orientation $\phi(t)/\phi(t=0)$ between particles (f). This behavior was observed for several pairs ($n = 10$) of particles of both sizes.

pair of arrows (perpendicular to each other) show the two principal directions of the stretch ratios. The directions of the stretch ratios are substantially variable throughout the cell. Also, in a given element the directions change rapidly with time (data not shown). Contrary to the one-dimensional directional freezing measured at the tissue level [23], the orientation of the vectors in these plots clearly indicates that local deformations at the cell level are varied in direction with respect to the freeze front. Similar results can be observed for another cell (b). For this cell, the

lower part of the cell flips as observed from the fluorescence images, which is probably caused by cell-matrix detachment at adhesion points.

A time-lapse image sequence of a representative cell is shown in Fig. 6(a). The top panel shows the fluorescence images indicating the gradual cell deformation, and the bottom panel surface plots show the corresponding spatiotemporal evolution of I_1 in the cell. The surface plots were generated from 33 triangular elements formed using the locations of 24 microbeads. The freeze

Fig. 5 Deformation in two representative cells (a) and (b) cultured on collagen gel. Arrow in (i) shows the direction of freeze front propagation. From the fluorescence images (i), (ii), and (iii) the cell deformation can be visualized. The surface plot (iv) shows the spatial distribution of I_1 at a given intermediate time. Surface plot for I_2 is presented in (v) at the same intermediate time. For the same time point, the directionality of the stretch ratios is presented in (vi) for some triangular elements. The pair of arrows represent mutually perpendicular principal stretch ratios (magnitude and directions found as eigenvalues and eigenvectors of left Cauchy-Green tensor B). The scale bar represents the unit stretch ratio with reference to the size of the arrows. Spatially they are variably oriented in the cell and different in magnitude from one part of the cell to the other.

front moves from left to right (arrow), touches the cell at around 2 s, deforms the cell, and then the freeze front sequentially passes over all elements of the cell. This results in a complex deformation pattern inside the cell. Deformation at various elements in the cell evolves temporally. The representative elements b, c, d (shown by arrows) in the cell show different deformation behavior. Element b undergoes compression followed by extension. This can be visualized by increased inter-particle distance in that region at later time instances. Element c undergoes similar deformation but significantly lower deformation than in b. Elements b and c start to deform even before the freeze front reaches the element. This may be caused due to fluid pressure induced from the left side of the cell through compression

of the extracellular matrix. Element d near the periphery of the cell deforms and indicates a sudden retraction-type movement. This element is compressed from 3 to 5 s, extends at 7 s and at 9 s the element comes back to a configuration it had at a previous instance. This sudden retraction may be caused by detachment of the cell from the matrix. These deformation phenomena are quantitatively presented in the bottom panel surface plots of I_1 . These plots show the spatiotemporal deformation pattern in the cell. As time passes, the cell deforms more, and the value of I_1 in the cell changes at most elements. Different elements in the cell deform differently and show various deformation features. Some elements become compressed ($I_1 < 2$) and some extend ($I_1 > 2$).

Fig. 6 Spatiotemporal intracellular deformation for a representative cell cultured on collagen gel. The top panel in (a) shows the deforming cell with internalized fluorescent microbeads. The arrow at bottom of the 3 s image shows the direction of freeze front propagation. Bottom panel in (a) shows the surface plot of I_1 for the same time points. The individual temporal evolution of I_1 for the three triangular elements indicated by b, c, d in (a) are presented in (b), (c), and (d).

Temporal histories of I_1 for the triangular elements b, c, and d indicated in (a) are presented in Figs. 6(b), 6(c), and 6(d), respectively. From these plots for individual elements, the deformation (compression/extension) as a function of time can be quantified. For all cases the freeze front touches the cell at 2 s (vertical line i). The element in (b) is first compressed when the freeze front approaches, reaches a minimum I_1 (vertical line ii at 4.7 s) and later, extension prevails after the freeze front hits the element, reaching a maximum I_1 value of 3.8. The element in (c) shows a similar pattern, but with significantly lower deformation than the element in (b), reaching maximum I_1 of 1.5. The element in (d) represents a case where detachment was observed. This detachment can be quantitatively captured from the temporal plots where I_1 undergoes a drastic change in value. The element undergoes compression and extension as the freeze front approaches. At 5.2 s the freeze front hits the element ($I_1 = 2.2$), and I_1 starts increasing steeply until 7.8 s, reaching a maximum $I_1 = 4.8$. After that within 0.8 s, I_1 steeply decreases to 2 characterizing the detachment feature.

5 Discussion

In this work, a new method, particle tracking deformetry, is developed to measure the spatiotemporal intracellular deformation for cells attached to a collagen gel matrix. The present results imply that cells in biological tissue may experience substantially higher mechanical loading than what is estimated from the tissue-level measurement. However, in biological systems, high deformation is abundantly encountered [36,37]. The maximum value of I_1 in some elements of cells are shown to reach around 12, characterizing six times the element deformation with respect to the ini-

tial state. Moreover, this deformation is observed to happen over only 2–3 s. This is equivalent to an area-based dilatation rate of nearly 0.1 s^{-1} . From previous cell image deformetry studies [7] performed at a similar freezing condition, the ECM deformation was shown to have a much lower value (area-based dilatation rate = 0.01 s^{-1}). This large mismatch in cell-level and matrix-level deformation suggests that the cell-matrix adhesion complex (CMAC) is under large mechanical loading and possible rupture, which implies a loss of functionality. In addition, the sudden detachments along the cell matrix adhesion points near the cell periphery, characterized by abrupt change in I_1 , may be associated with rupture of the CMAC. The deformation characterized by stretch ratios was variably directed in the cell in contrast to a one-dimensional deformation pattern [23] experienced at the tissue level. This may be due to the nonaffine attachment of the cells to the matrix through the CMAC [29,30]. Further research to simultaneously measure the extracellular and intracellular deformation is warranted.

One of the unique features of the present method is adopting the intracellular labeling instead of extracellular labeling adopted in other deformetry techniques. This unique feature enables direct measurements of cytoskeletal deformation without indirect calculation of the mechanical loading at the cell periphery. However, this is only possible when the intracellular particle movement indicates cytoplasmic deformation. Two possible scenarios when this assumption fails are (i) the particles are too small so that they move through the cytoskeletal structure or (ii) too many particles are internalized to hinder the cytoplasmic deformation. In this study it is maintained that these two scenarios do not occur. The microbeads used for the deformation measurement reflect the true intracellular deformation as they do not experience diffusive

movement in the cell. It was shown earlier that the microbeads are internalized in the endosomes of the cell and stay in the cytoskeletal structure [38]. It was also reported that the pore size of the cytoplasm is around tens of nm [39], and particles of comparable or larger size are trapped in the cytoskeletal structure and do not undergo diffusive motion. In a previous study to characterize flow-induced deformation in bone cells, the displacements of cell-bound microbeads were measured [40], where the cells were attached to a fibronectin-coated quartz slide. These previous studies, and the effect of particle size on deformation measurements reported in this work, confirm that microbead movement in the cell during cell deformation closely reflects cytoplasmic deformation in a short time interval. It was estimated that a cell generally uptakes nearly 50–60 microbeads, which comprises only a small volume fraction of the cell (0.3% of total cell volume). This confirms that the presence of microbeads minimally interferes with cellular mechanics.

The particle tracking deformetry (PTD) method for intracellular deformation measurement developed in this work has several distinct features compared to the extracellular deformation measurement performed earlier (cell image deformetry (CID)). First, this study measures the displacement and deformation by tracking the loci of tracking points (i.e., Lagrangian). On the contrary, CID measures displacement and deformation rate at given locations (i.e., Eulerian) by cross correlating interrogation windows of time-lapse images. Second, CID measures locally averaged quantities at the extracellular matrix level in certain sizes of interrogation windows. However, PTD measures subcellular deformation which is not averaged, as in the ECM level. Thus, the PTD measurement results can be substantially different from CID results due to these differences in measurement technique, as well as affine and nonaffine deformation in cell-matrix interactions [29,30].

In spite of its unique advantage, the present method needs to be further developed to probe the cellular/subcellular mechanics under physiologically relevant conditions. One of the major limitations is that the present results are a two-dimensional representation of a three-dimensional phenomenon. However, the depth of field for the current imaging was $0.65\text{ }\mu\text{m}$, which confirms that the planar computation was a good approximation. Assuming the cellular material is isotropic, the order of magnitude of the measured deformation values would still be similar as that experienced in 3D, and the qualitative distribution of spatiotemporal intracellular deformation revealed by this method should be the same as in 3D. Further development is necessary to image and track particles in the 3D domain and to subsequently analyze their deformation in order to measure actual intracellular spatiotemporal 3D deformation. The present work aims to develop a new measurement method of spatiotemporal intracellular deformation, and fibroblasts in collagen matrix were used as a model system. However, further characterization with other cell types would be beneficial to confirm the applicability of the developed method to various cell and tissue types. It is also required to measure the freezing-induced intracellular deformation for different freezing conditions and collagen matrix densities to understand the freezing-induced biomechanical processes inside cells. Further research will address these issues.

Though probing cell-level mechanical parameters has been actively pursued, so far the intracellular deformations are still very difficult to measure in both small length and time scales. Currently available methods to study cell mechanics include atomic force microscopy, optical tweezers, magnetic twisting cytometry, micropipette aspiration, shear flow in a channel, micropost-induced stretching, and microrheology [41–43]. However, the major limitations of those methods lie in the fact that all of them are applied for cells in isolation or on glass substrates, which is much different from the actual physiological environment where the cells are embedded in the complex ECM. The traction force microscopy technique estimates force and deformation at the cell surface from the ECM deformation pattern

through mathematical techniques, but is limited to application to cells in ECM [26,44]. The present method can provide the capability to measure rapid intracellular deformation, and is potentially applicable to cells in a matrix. The method described here can have broader application in probing intracellular deformation patterns and characterizing cellular mechanical parameters and mechanotransduction during physiological processes, namely cancer metastasis, vasculogenesis, wound healing, and matrix mechanics-dependent stem cell differentiation.

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